Secondary metabolites in different species of Brassica vegetables grown in greenhouse

Pablo Velasco*, Marta Francisco, Margarita Lema, María Elena Cartea

Department of Plant Genetics, Misión Biológica de Galicia (CSIC), PO Box 28, 36080 Pontevedra, Spain.
*Corresponding author: pvelasco@mbg.cesga.es

Introduction

In Galicia (northwestern Spain), different *Brassic*a species are used as leaf vegetable products for human and also for animal consumption. Kales (*Brassica oleracea acephala* group), cabbages (*B. oleracea capitata* group), leaf rape (*B. napus pabularia* group), and turnip tops and turnip greens (*B. rapa rapa* group) are the most important Brassica crops in this region. At the Misión Biológica de Galicia (CSIC, Spain), a collection of local varieties of these species is kept in a Germplasm Bank. The glucosinolate and phenolic profile, metabolites related with human health, has been previously studied in this collection (Padilla et al., 2007b; Velasco et al., 2007;2008; Cartea et al., 2008a; 2008b; Francisco et al., 2009) under field conditions. Nevertheless, due to the yield losses caused by several pests and the adverse weather conditions throughout the growing season, these crops are increasingly being cultivated under cover or greenhouse conditions. In these conditions, the content in secondary metabolites may be quite different and it has not been studied yet. For this reason, the objective of this work was to quantify the glucosinolate and phenolic content of four different crops: kale, cabbage, leaf rape and turnip greens grown under greenhouse conditions in two different times and to compare them with the same varieties studied in the field in different experiments.

Materials and Methods

Eight local varieties, currently kept in the Germplasm Bank at the 'Misión Biológica de Galicia' were evaluated in this study: two kale varieties (BOA) named 'MBG-BRS0468' and 'MBG-BRS0476' (*B. oleracea acephala*), two white cabbage varieties (BOC) named 'MBG-BRS0057' and 'MBG-BRS0074' (*B. oleracea capitata*), two leaf rape varieties (BN) named 'MBG-BRS0035' and 'MBG-BRS0063' (*B. napus pabularia*), and two turnip greens varieties (BR) named 'MBG-BRS0132' and 'MBG-BRS0401' (*B. rapa rapa*). The populations were planted in multipot-trays and seedlings were transplanted to bigger pots at the five or six leaves stage. Populations were evaluated in a randomized design with nine replications and 10 plants per replication. Leaf material was collected at two times (two and three months after transplanting). The third leaf of each plant was collected to extract and analyze glucosinolates and phenolic compounds. Greenhouse conditions were: light 16h/8h, maximum temperature 25 ºC, minimum temperature 10 ºC. The protocol for extraction and metabolite identification was followed as described by Francisco et al. (2009). Analyses of variance were made for each compound to compare species and varieties. Comparison of means was made by Fisher's protected least significant difference (LSD) at P=0.05 (Steel et al., 1997). All statistical analyses were made using SAS (SAS

Institute, 2007).

Results and Discussion

Twenty six compounds were found in the species studied, nine glucosinolates (GL), nine hydroxycinnamic acids (HA), and eight flavonoids (F). There were significant differences among species for total GL, HA and F ($P<0.05$; data not shown). BR was the species with the highest GL concentration followed by BN, BOC, and BOA. BR showed also the highest HA concentration followed by BOA, BN, and BOC. Finally, BN and BOA had the highest concentration of F, followed by BR and BOC (Table 1). Regarding the variation of secondary metabolites over sampling times, the concentration of F decreased for BR and BOC in 14% and 5% and increased for BN and BOA in 30% and 18%, respectively. HA concentration decreased a 9% in BR while increased in the other species from 15% in BN to 61% in BOA. Concentration of aliphatic GL increased in all species from 38% in BR to 93% in BN. In the other side, indolic GL decreased from 6% in BOC to 26% in BOA. The only aromatic GL decreased in all species from 1% in BOA to 26% in BOC.

When comparing the GL composition of these varieties grown in greenhouse conditions with the GL content of the same varieties grown in the field (Padilla et al., 2007b; Cartea et al., 2008a; 2008b), the greatest differences on GL content were found in BN and BOC. In BN proportions of aliphatic and indolic GL were 92% and 7% in field conditions, and 73% and 22% in greenhouse conditions. BOC showed more indolic GL (52%) in greenhouse than aliphatic GL (42%). In the field these varieties showed 43% of indolic GL and a 58% of aliphatic GL. Besides, total GL concentration of the species grown in the field was, as a mean, 2.5 times higher than in the greenhouse, showing a better development of these species in field conditions. For phenolic compounds, we could only compare BR species (Francisco et al., 2009). In field conditions, F concentration was a 40% of total phenolics and HA the 60%. In greenhouse conditions, F were the 27% of total phenolics and HA the 73%.

Conclusion

These results showed that different crop conditions results in different concentrations of secondary metabolites. As GL, HA and F are metabolites with implications in human health (i.e. anticarcinogenic or antioxidant), it is necessary to establish the best conditions in the cultivation of these species to obtain the highest concentration of healthy compounds.

Acknowledgements

Research was supported by the National Plan for Research and Development (AGL2009-09922) and the Excma. Diputación Provincial de Pontevedra. Authors thank Rosaura Abilleira for all the invaluable help in the laboratory work.

References

Cartea, M. E.; Rodriguez, V. M.; de Haro, A.; Velasco, P.; Ordas, A., Variation of glucosinolates and nutritional value in nabicol (Brassica napus pabularia group). Euphytica 2008, 159, (1-2), 111-122.

Cartea, M. E.; Velasco, P.; Obregon, S.; Padilla, G.; de Haro, A., Seasonal variation in glucosinolate content in Brassica oleracea crops grown in northwestern Spain. Phytochemistry 2008, 69, 403-10.

Francisco, M.; Moreno, D. A.; Cartea, M. E.; Ferreres, F.; García-Viguera, C.; Velasco, P., Simultaneous identification of glucosinolates and phenolic compounds in a representative collection of vegetable Brassica rapa. J Chromatogr A 2009, 1216, (38), 6611-9.

Padilla, G.; Cartea, M. E.; Velasco, P.; de Haro, A.; Ordas, A., Variation of glucosinolates in vegetable crops of Brassica rapa. Phytochemistry 2007, 68, (4), 536-545.

Velasco, P.; Cartea, M. E.; Gonzalez, C.; Vilar, M.; Ordas, A., Factors affecting the glucosinolate content of kale (Brassica oleracea acephala group). J. Agric. Food Chem. 2007, 55, (3), 955-962.

Velasco, P.; Soengas, P.; Vilar, M.; Cartea, M. E.; del Rio, M., Comparison of glucosinolate profiles in leaf and seed tissues of different Brassica napus crops. J. Am. Soc. Hort. Sci. 2008, 133, (4), 551-558.

Steel, R. D. G.; Torrie, J. H.; Dickey, D. A., Principles and procedures in statistics: a biometrical approach, 3rd ed. McGraw Hill, New York: 1997.

SAS Institute, The SAS System. SAS Online Doc. HTML Format Version Eight. SAS Institute Inc. Cary, North Carolina. USA.: 2007.

Table 1. Mean (µmol g^{-1} dw) for total glucosinolate content and phenolic compounds found in the four *Brassica* species evaluated at two sampling dates.

	Brassica rapa	*Brassica napus*	*Brassica oleracea acephala*	*Brassica oleracea capitata*
Aliphatic GL	25.90	11.23	4.59	4.94
Indolic GL	2.37	3.33	4.08	6.20
Aromatic GL	0.88	0.92	0.92	0.87
Total Glucosinolates	28.90	15.93	8.97	11.88
Total Hydroxycinnamic acids	28.79	20.45	22.78	14.37
Total Flavonoids	10.38	11.92	10.82	9.47
Total Phenolics	39.17	32.37	33.60	23.84